

## EFFECT OF ECLIPTA ALBA ON FRUCTOSE INDUCED HYPERTENSION IN ALBINO RATS

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## ABSTRACT

**Objective-** Hypertension is a common disorder rising in incidence once established treatment is obligatory. The causes, so also the drugs are many & usually the treatment is lifelong. Hence research is going on to find out a suitable & safe drug. Herbal products in this regard are worthy of investigation. Eclipta alba Hassk (Bhringaraja) is recently reported to have diuretic & antihypertensive activity in an ayurvedic study. This creates renewed interest for scientific evaluation of the claimed activity.

**Methods-** Hypertension was induced in male wistar albino rats by providing fructose (66%) in their diet for 15 days. Ethanolic extract of Eclipta alba (EEEE) leaves was administered in 100,200&400 mg/kg doses P.O.to different groups of rats for 7days starting from day 16<sup>th</sup> of fructose administration. The systolic, diastolic & mean arterial pressure (SBP, DBP, MAP) & heart rate(HR) were measured on day 7<sup>th</sup>,14<sup>th</sup>,21<sup>st</sup> & 28<sup>th</sup> day of administration standard & test drugs by non invasive BP system for rodents.

**Results-** A rise in blood pressure was found on day 16<sup>th</sup> of fructose diet. EEEA decreased the rise in B.P significantly in a dose dependent manner comparable to Quinapril.

**Conclusion-** Eclipta alba Hassk was found to possess significant antihypertensive activity in rat model. Further study in this line is necessary to pass its beneficial effects to clinical use.

**Keywords:** Eclipta alba, Antihypertensive effect, Quinapril, Albino rats

## INTRODUCTION

Hypertension currently is a common disorder growing in incidence globally particularly in developed countries. [1] Modern lifestyle might be one of the prime causes for such prevalence. Moreover hypertension is a multifactorial disease, accordingly the treatment is diverse. Once established, if left untreated results in serious cardiovascular, cerebral, renal and retinal complications. [2] Lot of drugs is already in use either alone or in combination. Drugs once started usually continued for lifelong. Hence research is still on, to find out a safe, cost effective and suitable drug for treatment of hypertension.

Herbal medicines (reserpine) have proved quite effective in lowering the increased blood pressure. The indigenous drugs are usually cheap & easily available, hence worthy of investigation as regards their effectiveness & safety for long term use.

Eclipta alba Hassk (Bhringaraja, Fam: Compositae) is a perennial shrub grown widely in the moist tropical countries. It is reported to have anthelmintic, antipyretic, anti-inflammatory, antihistaminic, hepatoprotective, expectorant properties [3, 4, 5, 7] and useful in disease of skin, spleen, stomatitis, toothache, hemicranias as well as vertigo. [3] Recently it's diuretic & anti hypertensive potentiality is claimed by the folklore as well as in an ayurvedic study [6] which creates a renewed interest for scientific evaluation of the same. Diuretics are useful in the treatment of a variety of diseases associated with abnormal retention of salt and water in the extracellular compartments of the body. Diuretic compounds as stimulate the excretion of water are potentially useful in many disorders including most of those exhibiting oedema such as congestive heart diseases, nephritis, toxemia of pregnancy, premenstrual tension, hypertension.[8] As an initial attempt, the diuretic potentiality of *Eclipta alba* was evaluated in our laboratory which revealed encouraging results. Therefore, the present study was undertaken to investigate the antihypertensive potentiality of Eclipta alba (if any) on albino rats.

## MATERIALS &amp; METHODS

## Principle of study

Hypertension was induced in rats by feeding them a fructose (66%) diet for 15 days. Antihypertensive effect of Eclipta Alba was assessed by observing the degree of lowering of BP in the hypertensive rats.

## Chemicals and Drugs

For the entire work, Quinapril and fructose were purchased from Sun Pharma, Mumbai and Merck Pharmaceuticals, Mumbai respectively in pure powdered form.

## Plant Material and Extraction

Leaves of Eclipta alba were purchased from the office of Range Officer, Padampur Forest Range, Nrushinghanath, district Bargarh, Odisha & authenticated by the Regional Plant Research Centre, Bhubaneswar. A sample of leaves was preserved in the same institute.

The coarsely powdered material of the shade-dried leaves was extracted with 95% ethanol in a Soxhlet apparatus in the laboratory of department of Pharmacology. The extract was concentrated under reduced pressure & stored in a refrigerator for further use.

## Acute toxicity study

Acute toxicity study was done according to OECD (Organization for Economic Co-operation and Development) Guideline, fixed dose method; with starting dose of 2000mg/kg body weight was adopted. Starting dose of 2000mg/kg (per oral) of each was given to 5 animals (albino rats), animals were kept for observation of behavioral change and death up to 72h.

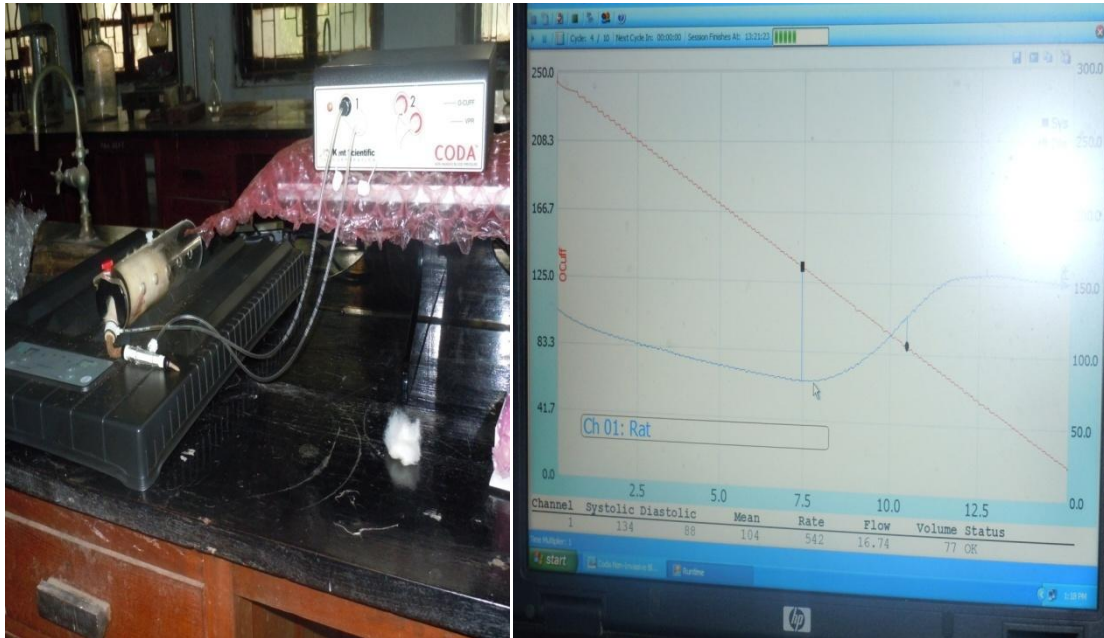
## Instrument

The instrument used for recording of blood pressure - Non-invasive BP instrument for rodents (NIBP system) was purchased from CODA, Kent Scientific, USA. The entire equipment set up includes magnetic animal holders connected with manual scanner, pulse amplifier & dual channel recorder.

## Principle of BP recording

Volume pressure sensor technology was adapted. Specially designed differential pressure transducer was utilized to measure the blood volume in the tail noninvasively. Different blood pressure parameters; viz; Systolic, Diastolic and Mean Arterial Pressure as well as heart rate (HR) were measured in each rat. The tail cuff method, without external preheating, was used to measure the above parameters. [11] Ambient temperature was kept at 30°C.

Non Invasive Blood Pressure Measurement System For Rodents



**Fructose induced hypertension**

30 male wistar albino rats weighing between 200-250 gms were used. Prior to the dietary manipulation all rats were fed standard rat chow, containing 60% vegetable starch, 11% fat & 29% protein and maintained on a 12 hour light/dark cycle. In addition, rats were acclimatized to the procedure of blood pressure measurement at 13.00 hour daily for one week. Methods of Hwang et al 1987 were followed. [9,10]

Following the training period, control rats (n=6) were continued on a diet of standard rat chow, where as the experimental groups (n=24) were kept on a diet containing 66% fructose, 12% fat and 22% protein. The electrolyte content of the two diets was reasonably comparable. The animals were continued on standard

rat chow or the fructose diet for 15 days. On day 16<sup>th</sup> food was removed at 08.00 hour. Rats were weighed & their blood pressure was measured by non- invasive BP system for rodents.

The blood pressure parameters were measured in the conscious state of the animals. The mean of 5 consecutive readings were taken as the recorded value of the SBP (systolic blood pressure), DBP (diastolic blood pressure), and MAP (mean arterial pressure) & HR (heart rate) of each rat for that day. The average of all parameters on the day 1(before starting the diet) & on the day 16 (day of experiment) were compared to assess the pattern of hypertension induced by fructose diet in rats (table-1) using unpaired student t'test. [9] Animals were allowed normal rat chow and water ad libitum from day 17<sup>th</sup> onwards.

**Table 1: Body weight & pattern of hypertension induced by fructose diet in albino rats**

Group	Treatment (po)	Body weight (gms)	SBP (mm Hg)	DBP	MAP	HR (beats/m)
Control	Normal chow diet	243.33 ± 3.33	121.83 ± 0.72	94.0 ± 0.62	100.16 ± 0.58	437.33 ± 0.55
Experimental	Fructose(66%) diet	249.16 ± 2.47	*148.95 ± 0.43	*108.08 ± 0.26	*126.03 ± 0.28	*469.91 ± 1.40

\*P<0.05, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, MAP: Mean Arterial Pressure, HR: Heart rate All values are mean of 5 observations + SEM

**Study of Antihypertensive effect of Ethanolic Extract of Eclipta alba (EAEA)**

The rats showing a raised SBP, DBP and MAP & HR on the 16<sup>th</sup> day, after being kept on fructose diet, were divided in to five groups of 6 animals each. The protocol of the study (Approved by IAEC) was designed in the following manner. Group 1 received normal saline (1ml), the vehicle and served as the control. Group 2 was administered with Quinapril 10 mg/kg [12] as the standard drug. Group 3, 4 & 5 received EAEA in doses of 100, 200 & 400 mg/kg as test drug. All the drugs were administered PO. The groups of animals received the respective treatment continuously for 21 days. The animals were weighed again and their SBP, DBP, MAP & HR were measured using the tail cuff method by the NIBP system on day 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> during the course of treatment & on day 28<sup>th</sup> after discontinuation of the treatment from day 21<sup>st</sup>.

Data were analyzed by one way ANOVA followed by Dunnet's multiple comparison tests. [13]

**OBSERVATIONS & RESULTS**

The observation indicated that there was no death in 2000mg/kg dose after 72hr.

A significant rise in SBP, DBP, MAP & HR was noticed on day 16<sup>th</sup> in rats kept on fructose diet for 15 days. No significant change in body weight was detected. Administration of EAEA continuously for 21 days to the hypertensive rats revealed (Table 2, 3, 4, 5) a decrease in systolic, diastolic, mean arterial pressure and heart rate in a dose dependent manner which was highly significant with 200 & 400 mg/kg doses. The onset of action was observed on day 7 post-dosing. The decline in all parameters was progressive as recorded on day 14 & 21 during continuous intake of the extract, peak effect being observed on day 21. A highly significant antihypertensive effect and bradycardia continued up to day 28, even if the drug was discontinued on day 21<sup>st</sup>. All the effects of EAEA were parallel to that of the standard drug quinapril except reduction of heart rate which was much less in comparison to quinapril.

**Table 2: Effect of EEEA on systolic BP of hypertensive rats on continuous therapy for 21 days & after a drug free period of 7 days.**

Treatment (PO)	Systolic BP (mm of Hg)				
	0	7	14	21	28#
NS (ml)	143.5 ± 1.31	144.83 ± 1.47	144.0 ± 1.15	142.5 ± 1.40	140.0 ± 1.63
Quinapril (10)	142.0 ± 1.23	***124.5 <sup>c</sup> ± 1.40	***99 <sup>c</sup> ± 2.40	***90.5 <sup>c</sup> ± 1.25	***92.3 <sup>c</sup> ± 1.47
EEEA(100)	145.0 ± 1.15	*139.16 <sup>c</sup> ± 1.10	***126.83 <sup>c</sup> ± 1.01	***124.5 <sup>c</sup> ± 0.95	***125.5 <sup>c</sup> ± 0.08
EEEA(200)	144.16 ± 1.40	**130.66 <sup>c</sup> ± 2.29	***122.16 <sup>c</sup> ± 0.87	***119.6 <sup>c</sup> ± 1.89	***120.3 <sup>c</sup> ± 1.20
EEEA(400)	145.16 ± 0.79	***122.66 <sup>c</sup> ± 0.843	***112.66 <sup>c</sup> ± 2.95	***109.6 <sup>c</sup> ± 1.74	***112 <sup>c</sup> ± 1.15

28#: value obtained after a drug free period of 7 days

Intra group comparison: Inter group comparison

a = (P<0.05), b = (P<0.01), c = (P<0.001)\* = (P<0.05), \*\* = (P<0.01), \*\*\* = (P<0.001)

**Table 3: Effect of EEEA on diastolic BP of hypertensive rats on continuous therapy for 21 days & after a drug free period of 7 days.**

Treatment (PO)	Diastolic BP (Mm of Hg)				
	0	7	14	21	28#
NS (ml)	110.5 ± 3.64	110.83 ± 3.38	111.33 ± 3.06	112.3 ± 1.89	110.6 ± 0.66
Quinapril (10)	108.6 ± 3.25	***90.33 <sup>c</sup> ± 1.20	***85.3 <sup>c</sup> ± 0.84	***84.33 <sup>c</sup> ± 1.40	***89.3 <sup>c</sup> ± 0.88
EEEA(100)	112 ± 2.0	**103.5 ± 3.22	**105.66 ± 1.30	***104.6 ± 1.76	***104.6 ± 1.76
EEEA(200)	111.3 ± 2.10	***94.66 <sup>c</sup> ± 1.33	***98.16 <sup>c</sup> ± 1.16	***95.3 <sup>c</sup> ± 1.33	***97 <sup>c</sup> ± 1.98
EEEA(400)	110 ± 3.05	***92.33 <sup>c</sup> ± 1.08	***88.16 <sup>c</sup> ± 1.19	***86.5 <sup>c</sup> ± 1.54	***87.5 <sup>c</sup> ± 1.40

28#: value obtained after a drug free period of 7 days

Intra group comparison: Inter group comparison

a = (P<0.05), b = (P<0.01), c = (P<0.001)\* = (P<0.05), \*\* = (P<0.01), \*\*\* = (P<0.001)

**Table 4: Effect of EEEA on mean arterial pressure of hypertensive rats on continuous therapy for 21 days & after a drug free period of 7 days.**

Treatment (PO)	Mean Arterial Pressure (Mm of Hg)				
	0	7	14	21	28#
NS (ml)	121.83 ± 2.09	122.16 ± 2.14	122.23 ± 1.89	122.4 ± 1.03	120.43 ± 2.44
Quinapril (10)	119.78 ± 2.15	***101.71 <sup>c</sup> ± 0.90	***89.9 <sup>c</sup> ± 0.41	***86.38 <sup>c</sup> ± 0.66	***90.31 <sup>c</sup> ± 0.89
EEEA(100)	122.98 ± 1.46	**115.38 <sup>b</sup> ± 2.07	***112.73 <sup>c</sup> ± 0.88	***110.93 <sup>c</sup> ± 0.91	***110.3 <sup>c</sup> ± 1.61
EEEA(200)	122.28 ± 1.50	***106.6 <sup>c</sup> ± 0.60	***106.16 <sup>c</sup> ± 0.60	***103.45 <sup>c</sup> ± 0.83	***104.7 <sup>c</sup> ± 1.50
EEEA(400)	121.45 ± 2.02	***102.45 <sup>c</sup> ± 0.62	***96.33 <sup>c</sup> ± 1.12	***94.23 <sup>c</sup> ± 1.02	***95.68 <sup>c</sup> ± 1.02

28#: value obtained after a drug free period of 7 days

Intra group comparison: Inter group comparison

a = (P<0.05), b = (P<0.01), c = (P<0.001)\* = (P<0.05), \*\* = (P<0.01), \*\*\* = (P<0.001)

**Table 5: Effect of EEEA on heart rate of hypertensive rats on continuous therapy for 21 days & after a drug free period of 7 days.**

Treatment (PO)	Heart rate (beats per min)				
	0	7	14	21	28#
NS (ml)	470.16 ± 5.72	469.16 ± 5.79	472.0 ± 8.85	470.0 ± 6.04	470.5 ± 2.94
Quinapril (10)	468.16 ± 11.77	***413.83 <sup>c</sup> ± 1.16	***288.0 <sup>c</sup> ± 5.06	***286.33 <sup>c</sup> ± 1.20	***300.66 <sup>c</sup> ± 2.66
EEEA(100)	470.66 ± 5.73	***444.66 <sup>c</sup> ± 1.76	***434.0 <sup>c</sup> ± 3.60	***432.0 <sup>c</sup> ± 2.52	***430.0 <sup>c</sup> ± 6.42
EEEA(200)	466.0 ± 4.56	***429.83 <sup>c</sup> ± 2.48	***411.0 <sup>c</sup> ± 1.88	***410.0 <sup>c</sup> ± 2.30	***410.33 <sup>c</sup> ± 2.02
EEEA(400)	469.0 ± 4.61	***418.33 <sup>c</sup> ± 1.58	***352.0 <sup>c</sup> ± 2.92	***349.66 <sup>c</sup> ± 1.20	***356.83 <sup>c</sup> ± 9.41

28#: value obtained after a drug free period of 7 days

Intra group comparison: Inter group comparison

a = (P<0.05), b = (P<0.01), c = (P<0.001)\* = (P<0.05), \*\* = (P<0.01), \*\*\* = (P<0.001)

## DISCUSSION

Addition of fructose (66%) to the diet for a minimum period of 15 days found to increase the blood pressure in the rodents that are not genetically destined to become hypertensive. The most accepted hypothesis proposes a strong relationship between insulin resistance/ hyperinsulinemia & the development of hypertension.[14,15] Several mechanisms viz. continued activation of sympathetic nervous system,[16] increased production &/or

activity of vasoconstrictors such as endothelin-1,[17] angiotensin II[18] or thromboxane A<sub>2</sub>[19] and impaired endothelium relaxation[20] are proposed to be the probable reasons of such hypertension.

The SBP, DBP, MAP & HR were recorded in the conscious animals in non invasive manner by a highly sensitive computerized digital blood pressure recording system manufactured by Kent Scientific, USA, which seems to give accurate readings without any autonomic disturbances.

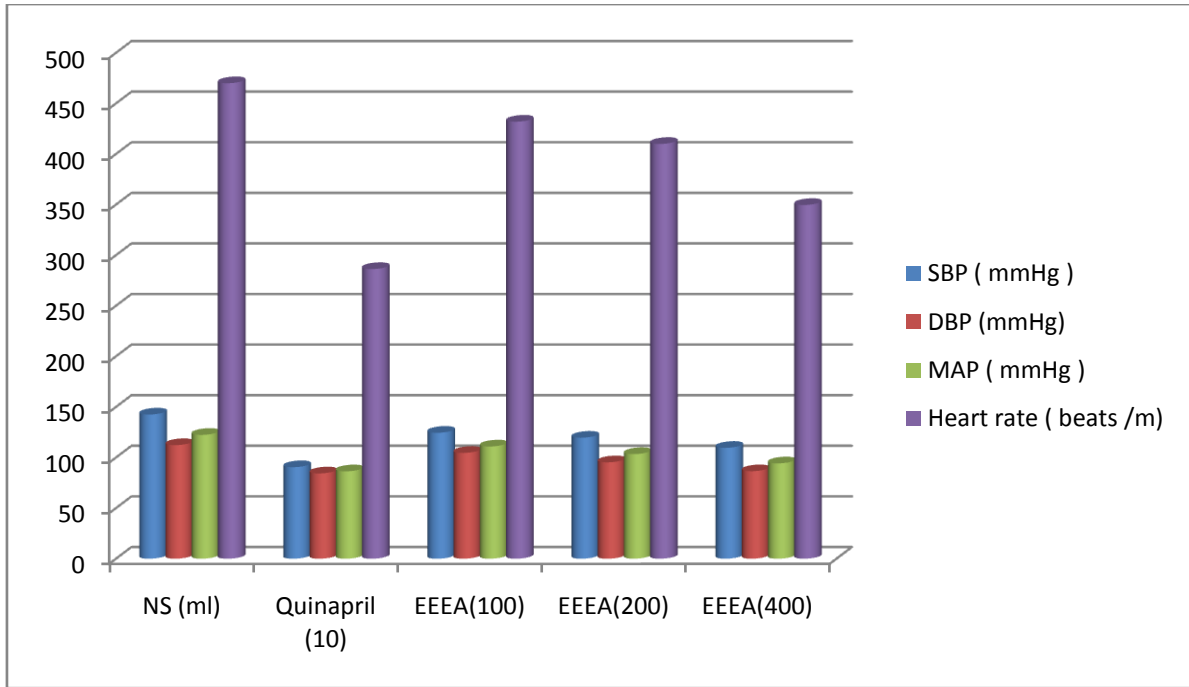


Fig. 1: Effect of EEEA on fructose induced hypertension in rats on 21<sup>st</sup> day (during treatment)

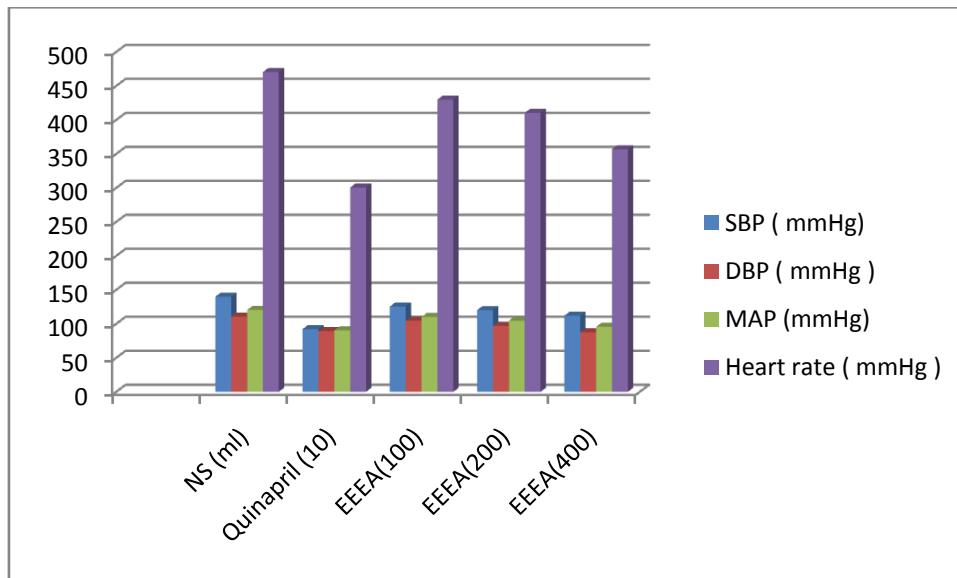


Fig. 2: Effect of EEEA on fructose induced hypertension in rats on 28<sup>th</sup> day, 7 days after discontinuation of treatment

Ethanol extract of *Eclipta alba* exhibited antihypertensive effect in the form of a significant lowering in systolic, diastolic and mean arterial pressure after continued administration for 7 days. The heart rate was also decreased significantly in comparison to control group. The reduction in blood pressure and heart rate was dose dependent (vide table 2-5 & figure 1), progressive and the peak effect was observed after three weeks of continuous therapy. Since continued activation of sympathetic nervous system and production of vasoconstrictor viz. angiotensin II is postulated in pathogenesis of fructose induced hypertension, quinapril, the ACE inhibitor was selected as the standard drug. Persistence of highly significant antihypertensive effect was noticed even after cessation of dosing 7 days earlier. This suggests absence of rebound phenomenon after withdrawal of the test drug which is an advantage in therapy of hypertension. The reduction in heart rate was much less in comparison to quinapril which might be due

to a difference in mechanism of action between the two. Results of present study are in agreement with the observation of Rangineni et al, 2007. [6]

Hypertension is common debilitating illness among the people in both developed and developing countries. The screening of various plants according to their traditional uses and nutritional value based on their therapeutic value leads to discovery of newer and safer alternative for management of hypertension. [21] Herbal products usually contain many active ingredients which either alone or in combination is responsible for the observed antihypertensive effect. Such an active ingredient, culumbin has been reported to exhibit significant antihypertensive effect. [3] Besides the diuretic potentiality of EEEA has been detected earlier in a separate study by the present authors which might in part be responsible for the antihypertensive effect.

## CONCLUSIONS

*Eclipta alba* Hassk exhibited significant antihypertensive activity against fructose induced hypertension in rats. Further study including phytochemical analysis, methods designed to explore its mechanism of action and toxicity tests etc. are necessary to pass on its beneficial effects to clinical use.

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## REFERENCES

- Goldblatt H, Lynch J, Hanzal RF, Summerville WW. Studies of experimental hypertension: 1. Production of persistent elevation of systolic blood pressure by means of renal ischemia. *J Exp Med*, 1934; 59:347-79.
- Rang HP, Dale MM, & Ritter JM. *Pharmacology* 4<sup>th</sup> ed, Churchill Livingstone, London; 1999.
- Chopra RN, Nayar SL, Chopra IC. *Glossary of medicinal plants*, CSIR publication, New Delhi, 1966,104
- Mehra PN, Nanda SS. Pharmacognosy of *Bhringaraja*. Antihepatotoxic drug of Indian origin. *Indian J Pharmacol*, 1968; 30:284.
- Chandra T, Sadique J and Soma Sundaram S. Effect of *Eclipta alba* on inflammation and liver injury. *Fitoterapia*, 1987;58(1):23-3
- Rangineni V, Sharada D, Saxena S. Diuretic, Hypotensive, and Hypocholesterolemic effects of *Eclipta alba* in Mild Hypertensive Subjects. *Journal of Medicinal Food*. March 1, 2007, 10(1): 143-148
- Pharmacopoeia of India*, Edition 1985
- K. Amala, A.saraswathy, S. Amerjothy. Hepatoprotective and diuretic activities of alcoholic and aqueous extracts of *Epilates pygmaea* dc. (ASTERACEAE) *Int J Pharm Pharm Sci*, 2013;5:Suppl 2, 502-506
- Hwang IS, Ho H, Hoffman B and Reaven GM. Fructose-induced insulin resistance and hypertension in rats. *Hypertension*, 1987; 10:512-516.
- Hwang IS, Hwang WC, Wu JN, Reaven GM. Effect of fructose induced hypertension on the rennin-angiotensin-aldosterone system & arterial natriuretic factor. *Am J Hypertens*, 1989; 2:424-7.
- Bunang RD, Butterfield J. Tail cuff blood pressure measurement without external preheating in awake rats. *Hypertension*, 1982; 4:898-903.
- Uchida T, Okumura K, Ito T, Kamiya H, Nishimoto Y, Yamada M, Tomida T, Matsui H, Hayakawa T. Quinapril treatment restores the vasodilator action of insulin in fructose-hypertensive rats. *Clin Exp Pharmacol Physiol*. 2002 May-Jun;29(5-6):381-5
- Jing-Xin Li, Bing Xue, Qing Chai, Zhi-Xiang Liu, Ai-Ping Zhao & Lian-Bi Chen. Antihypertensive effects of total flavonoid fraction of *Astragalus complantus* in hypertensive rats. *Chinese J of Physiol*, 2005; 48(2):101-6.
- Reaven GM. Insulin resistance, hyperinsulinemia, and hypertriglyceridemia in the etiology and clinical course of hypertension. *Am J Med*, 1991;90:7S-12S
- Reaven GM. Relationship between insulin resistance and hypertension. *Diabetes Care*, 1991; 14 (suppl 4):33-38.
- Penicaud L, Berthault MF, Morin J, Dubar M, Ktorza A, Ferre P. Rilmenidine normalizes fructose-induced insulin resistance and hypertension in rats. *J Hypertens Suppl*, 1998; 16:S45-49.
- Cosenzi A, Bernobich E, Plazzotta N, Seculin P, Bellini G. Bosentan reduces blood pressure and the target-organ damage induced by a high-fructose diet in rats. *J Hypertens* 1999; 17:1843-1848.
- Iimura O, Shimamoto K, Matsuda K, Masuda A, Takizawa H, Higashiura K *et al*. Effects of angiotensin receptor antagonist and angiotensin converting enzyme inhibitor on insulin sensitivity in fructose-fed hypertensive rats and essential hypertensives. *Am J Hypertens*, 1995; 8:353-357.
- Galipeau D, Arikawa E, Sekirov I, McNeill JH. Chronic thromboxane synthase inhibition prevents fructose-induced hypertension. *Hypertension*, 2001; 38:872-876.
- Katakam PV, Ujhelyi MR, Hoenig ME, Miller AW. Endothelial dysfunction precedes hypertension in diet-induced insulin resistance. *Am J Physiol*, 1998; 275: R788-792.
- Umang H. Joshi, Tejas H. Ganatra, Tusharbindu R. Desai, Pravin R. Tirgar. Evaluation of antihypertensive activity of *Evolvulus alsinoides* in adrenaline Induced hypertensive rats. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2012; 4(4):194-198